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Antimelanogenic and antioxidant effects of trimethoxybenzene derivatives: methyl 3,4,5-trimethoxybenzoate, ethyl 3,4,5-trimethoxybenzoate, methyl 3,4,5-trimethoxycinnamate, and ethyl 3,4,5-trimethoxycinnamate

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Abstract In this study, derivatives of trimethoxybenzene were investigated as inhibitors of melanogenesis. We examined the effects of methyl 3,4,5-trimethoxybenzoate (MTB), ethyl 3,4,5-trimethoxybenzoate (ETB), methyl 3,4,5-trimethoxycinnamate (MTC), and ethyl 3,4,5-trimethoxycinnamate (ETC). First, the inhibitory effects of these agents on melanin production were evaluated using α-melanocyte-stimulating hormone (α-MSH)-stimulated B16F10 melanoma cells. We found that all derivatives decreased α-MSH-induced melanin production in B16F10 melanoma cells; ETC showed a strong inhibitory effect at half of the concentration of the other derivatives. As tyrosinase is considered a key enzyme of melanogenesis, we also examined whether the derivatives inhibited tyrosinase activity. MTC and ETC reduced mushroom tyrosinase activity and expression levels of α-MSH-induced B16F10 cellular tyrosinase protein. Inhibitory effects of all derivatives on α-MSH-induced B16F10 cellular tyrosinase activity were shown in a dose-dependent manner. Additionally, the derivatives were exposed to diphenylpicryl-hydrazyl free radical to examine their antioxidant characteristics. All derivatives showed considerable antioxidant activity, which was 2-fold higher than that of arbutin. In conclusion, the trimethoxybenzene derivatives, including MTB, ETB, MTC, and ETC exerted anti-melanogenic and antioxidant effects on α-MSH-stimulated melanogenesis, demonstrating their potential for use as novel hypopigmenting agents and antioxidants.

Keywords Alpha-MSH · Antioxidants · Benzoates · Cinnamates · Melanocytes · Skin pigmentation

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Introduction

Melanin is a pigment produced by epidermal melanocytes and malignant melanoma cells in response to ultraviolet light and is responsible for developing various skin colors among different ethnic groups [1,2]. Although melanin is essential for protecting the human skin from severe ultraviolet light-mediated injury, excessive or unrestrained melanin production induces hyper-pigmentation such as freckles and melasma.

During melanin biosynthesis, also known as melanogenesis, tyrosinase functions as a critical enzyme by catalyzing the first rate-limiting step in which 3,4-dihydroxyphenylalanine (L-DOPA) is hydrolyzed to dopaquinone [3]. This enzymatic oxidation activity of tyrosinase is highly correlated with the pigment level in cultured melanocytes [4,5]. Moreover, it is widely accepted that tyrosinase's total protein or mRNA level is correlated with melanin content. One study demonstrated that α-melanocyte-stimulating hormone (α-MSH)-induced tyrosinase activation is associated with increased tyrosinase protein levels [6]. This suggests that regulation of tyrosinase activity represents a fundamental approach to preventing the development of hyperpigmentation.

Melanogenesis is also affected by several reactive oxygen species (ROS), including hydrogen peroxide, which serves as a mediator that controls the activation of transcription factors of signaling pathways [7,8]. As ROS represents critical components in melanogenesis regulation, ROS scavengers are used to decrease the level of UV-induced melanin production [9]. Previous studies of ROS scavengers such as N-acetyl cysteine, which downregulates UVB-induced α-MSH showed that treating melanocytes with ROS scavengers could induce depigmentation [10].

Skin-whitening agents are highly demanded in the global cosmetic industry, particularly in Asian countries [11]. Although many natural-derived skin-whitening agents are commercially available, it is unlikely to utilize practically in industrial applications due to their structural instability [12]. For instance, arbutin is a natural product extracted from the bearberry plant and can be produced by *in vitro* culture of Schisandra chinensis [13]. However, natural derivatives of arbutin have unstable structures that can release the highly toxic compound hydroquinone [14]. L-Ascorbic acid is easily oxidized and susceptible to heat [15]. Because of the limitations of existing tyrosinase inhibitors, novel tyrosinase inhibitors with relatively better stabilities are needed.

Trimethoxybenzene, which has a structural similarity with benzoates and cinnamates, is a natural flavonoid found in plants and functions as an antioxidant and an inflammasome inhibitor [16]. It is widely accepted that antioxidative polyphenol chemicals can potentially be hypopigmenting agents due to their structural correlation between tyrosinase and the impact of oxidative stress on melanogenesis, as previously mentioned [17-20]. However, effective antioxidative compounds without structural modifications quickly degrade by external environmental factors such as light [21]. According to Kim et al. [22], methyl gallate, which has significant structural similarity with trimethoxybenzene, decreased the protein expression of microphthalmia-associated transcription factor (MITF), which regulates several enzymes along melanogenesis. Nonetheless, the dermatological application of other derivatives of trimethoxybenzene has not been widely examined. Herein, we investigated the potential anti-melanogenic and antioxidant effects of the following derivative: methyl 3,4,5-trimethoxybenzoate (MTB), ethyl 3,4,5-trimethoxybenzoate (ETB), methyl 3,4,5-trimethoxycinnamate (MTC), and ethyl 3,4,5-trimethoxycinnamate (ETC).

Materials and Methods

Reagents and antibodies

Dulbecco's phosphate-buffered saline (PBS) was purchased from Cytiva (Logan, UT, USA). The 2N sodium hydroxide standard solution, dimethyl sulfoxide (DMSO), and sodium carbonate were purchased from Daejung (Siheung-si, Korea). Trimethoxybenzoic acid, trimethoxycinnamic acid, α-MSH, sodium phosphate dibasic anhydrous, sodium phosphate monobasic anhydrous, tyrosinase derived from mushroom, and L-DOPA were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,2-Diphenyl-1-picrylhydrazyl free radical (DPPH) was purchased from Alfa Aesar (Thermo Fisher Scientific, Waltham, MA, USA). Cell lysis buffer (10×) was purchased from Cell Signaling Technology (Danvers, MA, USA). Skim milk was purchased from BD Biosciences (Franklin Lakes, NJ, USA). Western enhanced chemiluminescence substrates and Triton X-100 were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Antibodies against tyrosinase and horseradish peroxidase-conjugated anti-rabbit immunoglobulins were from Santa Cruz Biotechnology (Dallas, TX, USA). Antibodies against heat shock protein 90 (HSP90) were obtained from Abcam (Cambridge, UK).

General procedure for preparation of derivatives

For the preparation of MTB or CTB, 3,4,5-trimethoxybenzoic acid (0.1 mol, 21.2 g) was dissolved in 150 mL of DMSO. The solution was stirred and heated at 50 °C in 500 mL round bottom flask. Sodium carbonate (0.2 mol, 21.2 g) was slowly added to the reaction solution, followed by methyl bromide (0.15 mol, 127.5 g) for MTB or ethyl bromide (0.15 mol, 154.5 g) for ETB. The reaction mixture was stirred for 4 h. After completion of the reaction, 150 mL of distilled water was poured into the reaction mixture, and the reaction flask was cooled to room temperature. The reaction flask formed the precipitation depending on temperature, which was getting down. The white precipitate was filtered with a vacuum to obtain the product.

For the preparation of MTC or ETC, 3,4,5-trimethoxycinnamic acid (0.1 mol, 23.8 g) was dissolved in 150 mL of DMSO. The solution was stirred and heated at 50 °C in 500 mL round bottom flask. Sodium carbonate (0.2 mol, 21.2 g) was added to the reaction solution, followed by methyl bromide (0.15 mol, 127.5 g) for MTC or ethyl bromide (0.15 mol, 154.5 g) for ETC. (0.15 mol, 127.5 g) slowly. The reaction mixture was stirred for 4 h. After completion of the reaction, 150 mL of distilled water was poured into the reaction mixture, and the reaction flask was cooled to room temperature. The reaction flask formed the precipitation depending on temperature, which was getting down. The white precipitate was filtered with a vacuum to obtain the product.

Cell culture

B16F10 melanoma cells were obtained from the Korean Cell Line Bank (Seoul, Korea) and incubated at 37 °C in a 5% CO_2 incubator in Dulbecco's Modified Eagle Medium (Cytiva) containing 10% fetal bovine serum (Thermo Fisher Scientific) and 1% antibiotic-antimycotic mixture (Sigma-Aldrich).

Cell viability

Cell viability was determined via the Aqueous One Solution Cell Proliferation Assay (MTS). B16F10 melanoma cells were cultured in serum-free media at a density of 5×10^4 cells/well in 24-well plates and incubated overnight. The cells were treated with or

without MTB, ETB, MTC, ETC, or arbutin at different concentrations. After 24, 48, or 72 h incubation, CellTiter 96® solution (MTS solution; Promega, Madison, WI, USA) was added, and the cells were incubated at 37 °C in a 5% CO_2 incubator for 3 h and transferred to 96-well plates. The absorbance was measured at 475 nm using a SpectraMax 190 plate reader (Molecular Devices, Sunnyvale, CA, USA).

Measurement of inhibition of mushroom-derived tyrosinase activity

The direct enzyme-inhibitor interactions between tyrosinase and derivatives can be explored by spectrophotometrically measuring enzymatic activity of mushroom tyrosinase (23-24). In 96-well plates, MTB, ETB, MTC, or ETC (0, 2, 5, or 10 μg/mL, respectively), or arbutin (125 μg/mL) was mixed with 10 mM L-DOPA and mushroom tyrosinase (200 U/mL) in sodium phosphate buffer (100 mM; pH 6.8). The plates were incubated at room temperature for 2 min, and absorbance was measured at 475 nm using SpectraMax 190 plate reader. Inhibition of mushroom tyrosinase activity was calculated using the following equation:

$$\text{Mushroom tyrosinase activity inhibition (\%)} = \left[1-\left\{1-\frac{B-C}{A-D}\right\}\right]\times 100$$

where A is the absorbance of the 200 U/mL mushroom tyrosinase treatment group, B is the absorbance of the 200 U/mL mushroom tyrosinase and derivative group, and C is the absorbance of the derivative treatment group, and D is the absorbance of sodium phosphate buffer without treatment with mushroom tyrosinase or derivative.

Measurement of intracellular and extracellular melanin

The *in vitro* intracellular and extracellular melanin content was measured to evaluate the attenuation of α-MSH-stimulated melanin production [23]. B16F10 melanoma cells were cultured in serum-free media at a density of 5×10^4 cells/well in 24-well plates and incubated overnight. The cells were treated with or without MTB, ETB, MTC, or ETC (0, 2, 5, or 10 μg/mL, respectively), or arbutin (125 μg/mL) as the positive control in the presence or absence of α-MSH (500 ng/mL). After 24 h incubation, the culture medium was collected, and the absorbance was measured at 405 nm using SpectraMax 190 plate reader to determine the extracellular melanin content. To evaluate the intracellular melanin content, after 72 h incubation with or without the derivatives or arbutin, the cell suspensions were disrupted using cell lysis buffer containing protease inhibitor. After washing with PBS and centrifugation, the pellets were dissolved in 1 N NaOH containing 10% DMSO. The solution was heated in an oven at 60 °C for 2 h, and the absorbance was measured at 405 nm using SpectraMax 190 plate reader to determine the intercellular melanin content.

Measurement of inhibition of intracellular tyrosinase activity in B16F10 melanoma cells

Inhibition of *in vitro* intracellular tyrosinase activity was measured as described by Molagoda et al. [25], with minor modifications. B16F10 melanoma cells were cultured in serum-free media at a density of 5×10^4 cells/well in 24-well plates and incubated overnight. The cells were then treated with and without MTB, ETB, MTC, or ETC (0, 2, 5, and 10 μg/mL, respectively), or arbutin (125 μg/mL) in the presence or absence of α-MSH (500 ng/mL). After 72 h of incubation, the cell pellets were disrupted using PBS containing 1% Triton X-100, and the cell extracts were mixed with freshly prepared 10 mM L-DOPA. The absorbance was measured at 475 nm using SpectraMax 190 plate reader.

Protein extraction and western blotting

B16F10 melanoma cells were cultured in serum-free media at 5×10^4 cells/dish in 100 dishes and incubated overnight. The cells were then treated with MTB, ETB, MTC, or ETC (0, 2, 5, and 10 μg/mL, respectively) in the presence or absence of α-MSH (500 ng/mL). After 72 h incubation, the cells were disrupted using cell lysis buffer containing protease inhibitor cocktails and centrifuged. The protein content was quantified using a Bio-Rad protein assay. An equal amount of protein (30 μg/well) was separated using 10% polyacrylamide gels and transferred onto polyvinylidene fluoride membranes. Immunoblotting was performed using primary antibodies against tyrosinase and HSP90. Immunoblotting was performed using horseradish peroxidase-conjugated anti-rabbit immunoglobins, and the results were visualized using enhanced chemiluminescence substrates. The protein expression levels were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Measurement of DPPH free radical-scavenging activity

To evaluate the reduction of oxidative stress, the antioxidant activity of the samples was measured using a method based on DPPH free radical-scavenging activity as described previously, with minor modifications [1,26]. First, preliminary experiments were performed using ETB (0, 2, 5, and 10 μg/mL) under various DPPH reaction times and initial concentrations (0, 20, 50, and 100 μmol/L). The absorbance was measured at 517 nm to determine the optimal condition. Following optimization, DPPH (20 μmol/L) was added to the derivatives (0, 2, 5, and 10 μg/mL) and incubated at room temperature for 80 min. The absorbance was measured at 517 nm using SpectraMax 190 plate reader. Arbutin (125 μg/mL) was used as a positive control. DPPH scavenging activity was estimated using the following equation:

$$\text{DPPH scavenging activity (\%)} = \left[1-\left\{1-\frac{B-C}{A-D}\right\}\right]\times 100$$

where A is the absorbance of DPPH solution not treated with a

derivative and B is the absorbance of the DPPH solution treated with a derivative.

Results

Anti-melanogenic effects of trimethoxybenzene derivatives on B16F10 melanoma cells without cytotoxicity

The cytotoxicity of MTB, ETB, MTC, and ETC in B16F10 melanoma cells was evaluated to determine the non-cytotoxic conditions. Cell viability was estimated via MTS assays at 24-h intervals and was estimated as approximately >80% (expressed as a percentage of the viability of the control) following treatment with all derivatives at concentrations of 2, 5, 10, 25, 50, and 100 μg/mL for 24, 48, and 72 h, respectively (Fig. 1). This suggests that a relatively higher concentration (≥25 μg/mL) induces cytotoxicity in B16F10 cells, in agreement with previous data [27]. Additionally, the cytotoxic effect of arbutin was measured as a positive control at various concentrations. At concentrations of >250 μg/mL, arbutin treatment considerably decreased the cell population.

To evaluate the inhibitory effects of MTB, ETB, MTC, and ETC on melanin production, the intracellular and extracellular melanin content in B16F10 cells was determined after 72 h incubation with or without MTB, ETB, MTC, and ETC (0, 2, 5, and 10 μg/mL, respectively) in the presence or absence of α-MSH (500 ng/mL). The α-MSH-stimulated melanin production rate was calculated as a percentage of the melanin content in the untreated group (0 μg/mL). A considerable reduction in extracellular and intracellular melanin content was observed after treatment with MTB, ETB, and MTC in a dose-dependent manner. The extracellular melanin content decreased to 86.9±2.1, 88.4±1.6, and 85.7±3.5% upon treatment with MTB, ETB, and MTC, respectively. ETC at a concentration of 5 μg/mL showed the strongest inhibitory effect and decreased the α-MSH-induced extracellular melanin production rate to 81.7±4.6%. The intracellular melanin content showed considerable reduction after the treatment of MTB, ETB, MTC, and ETC likewise (Fig. 2).

Inhibitory effects on catalytic activity and protein expression of tyrosinase

The catalytic activity of tyrosinase can be estimated by quantifying monophenolase activity using L-DOPA rather than L-tyrosine as a substrate [28]. Mushroom-derived tyrosinase is most commonly used in an *in vitro* assay for evaluating inhibitors that target tyrosinase to develop skin-brightening agents [15]. MTB, ETB, MTC, and ETC (0, 2, 5, and 10 μg/mL) were treated with 110 U of mushroom tyrosinase with 10 mM L-DOPA, and the DOPA oxidase activity of mushroom tyrosinase was estimated as a percentage compared to the control. This method did not reveal significant inhibitory effects of MTB and ETB on mushroom tyrosinase activity (Fig. 3A).

In the intracellular melanogenesis signaling pathway, various factors regulate tyrosinase activation and melanin synthesis [29]. Accordingly, we evaluated the effect of MTB, ETB, MTC, ETC on α-MSH-stimulated cellular tyrosinase in B16F10 cell lysates (Huang et al., 2014). Particularly, B16F10 cells were treated with MTB, ETB, MTC, and ETC (0, 2, 5, and 10 μg/mL) in the presence or absence of α-MSH (500 ng/mL) for 72 h. The cell lysates were then extracted and mixed with L-DOPA. The monophenolase activity of α-MSH-stimulated cellular tyrosinase was calculated as a percentage in the untreated group (0 μg/mL). Tyrosinase activity was reduced by MTB, ETB, MTC, and ETC in a dose-dependent manner (Fig. 3B).

Western blotting was performed to determine the effects of the derivatives on tyrosinase protein expression levels. Precisely, the amount of protein in B16F10 cells was quantified after treatment with MTB, ETB, MTC, ETC (0, 2, 5, and 10 μg/mL) in the presence or absence of α-MSH (500 ng/mL) for 72 h. GAPDH was used as a control to normalize the tyrosinase protein

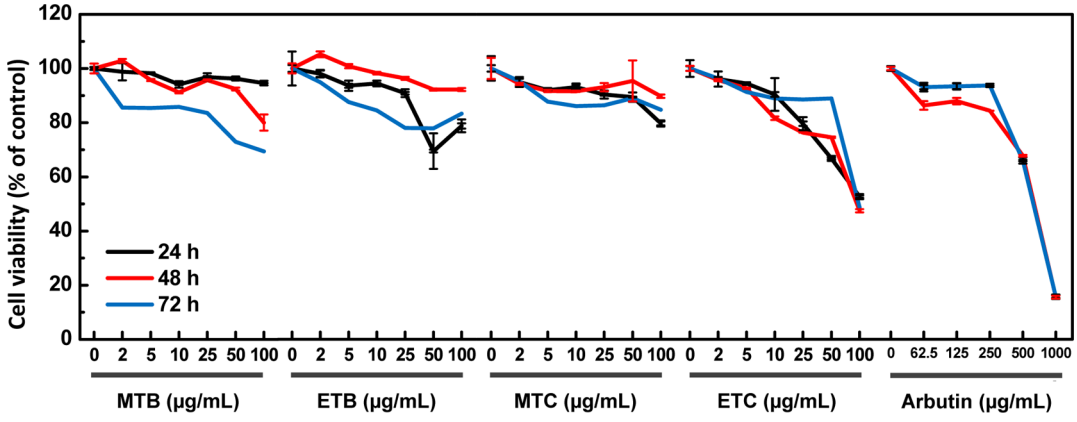

Fig. 1 Cell viability via MTS assays. B16F10 melanoma cells were incubated with MTB, ETB, MTC, ETC, and arbutin, as a positive control, for 24, 48, and 72 h. Control, untreated group; MTB, methyl 3,4,5-trimethoxybenzoate; ETB, ethyl 3,4,5-trimethoxybenzoate; MTC, methyl 3,4,5-trimethoxycinnamate; ETC, ethyl 3,4,5-trimethoxycinnamate

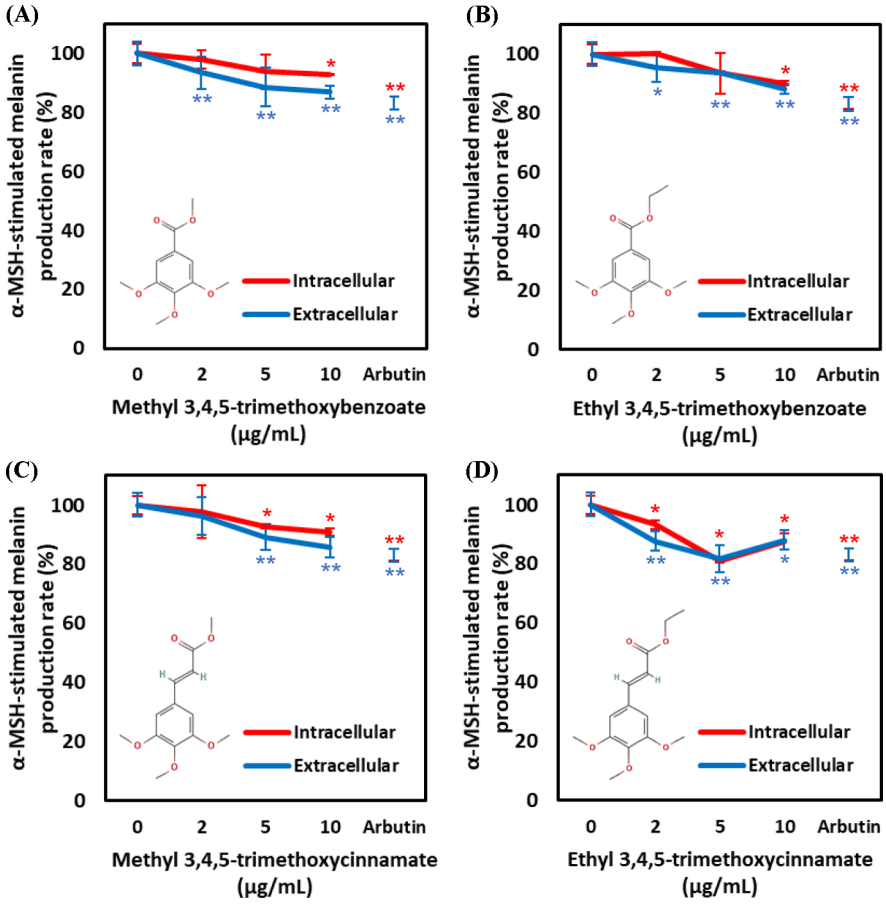

Fig. 2 Inhibitory effects of (A) MTB, (B) ETB, (C) MTC, and (D) ETC on α-MSH-induced melanin. Statistical significance was compared with "0" group (*p <0.05, **p <0.01). MTB, methyl 3,4,5-trimethoxybenzoate; ETB, ethyl 3,4,5-trimethoxybenzoate; MTC, methyl 3,4,5-trimethoxycinnamate; ETC, ethyl 3,4,5-trimethoxycinnamate; α-MSH, alpha-melanocyte-stimulating hormone

expression, and the expression levels were analyzed using ImageJ software (Fig. 4). The protein expression levels of tyrosinase in B16F10 cells were considerably reduced after treatment with ETB (10 μg/mL) and MTC (10 μg/mL) compared to those after treatment with arbutin (125 μg/mL); ETC decreased tyrosinase protein expression levels in a dose-dependent manner.

Antioxidant activity

The antioxidant activity of MTB, ETB, MTC, ETC was evaluated using DPPH, a stable radical that can react with antioxidants [25]. Arbutin (125 μg/mL), known as an antioxidant reducing ROS levels and intracellular production of cellular hydroxyl radical, was used as a positive control [30,31]. The DPPH concentration (Fig. 5A) and reaction time (Fig. 5B) were evaluated as independent variables following based on the results of preliminary experiments [26,27]. We thereby conditioned the variables as 20 μmol/L for DPPH concentration and 80 min for reaction time, respectively considering the suitable amount of free radicals for derivative concentrations and the sufficient time to determine the scavenging effect of derivatives, which related to the saturated region of scavenging activity (5 μg/mL, Fig. 5B). Subsequently, MTB, ETB, MTC, ETC (0, 2, 5, and 10 μg/mL, respectively) were mixed with DPPH (20 μmol/L), and the DPPH scavenging activity was measured after 80 min. As shown in Fig. 5C, MTB, ETB, MTC, and ETC considerably reduced the levels of DPPH free radicals in a dose-dependent manner, and all derivatives at 10 μg/mL reduced free radicals, showing approximately 2-fold more potent effects compared to that of arbutin (125 μg/mL).

Discussion

We evaluated derivatives of trimethoxybenzene to determine their suitability for use as skin-whitening agents by measuring cytotoxicity, melanin content, inhibitory effects on tyrosinase activity, and protein expression. The results were compared with those of a well-known inhibitor of tyrosinase, arbutin.

MTB, ETB, and MTC inhibited α-MSH-induced melanin production in a dose-dependent manner, whereas ETC showed a substantial dose-independent reduction in activity. Arbutin, a

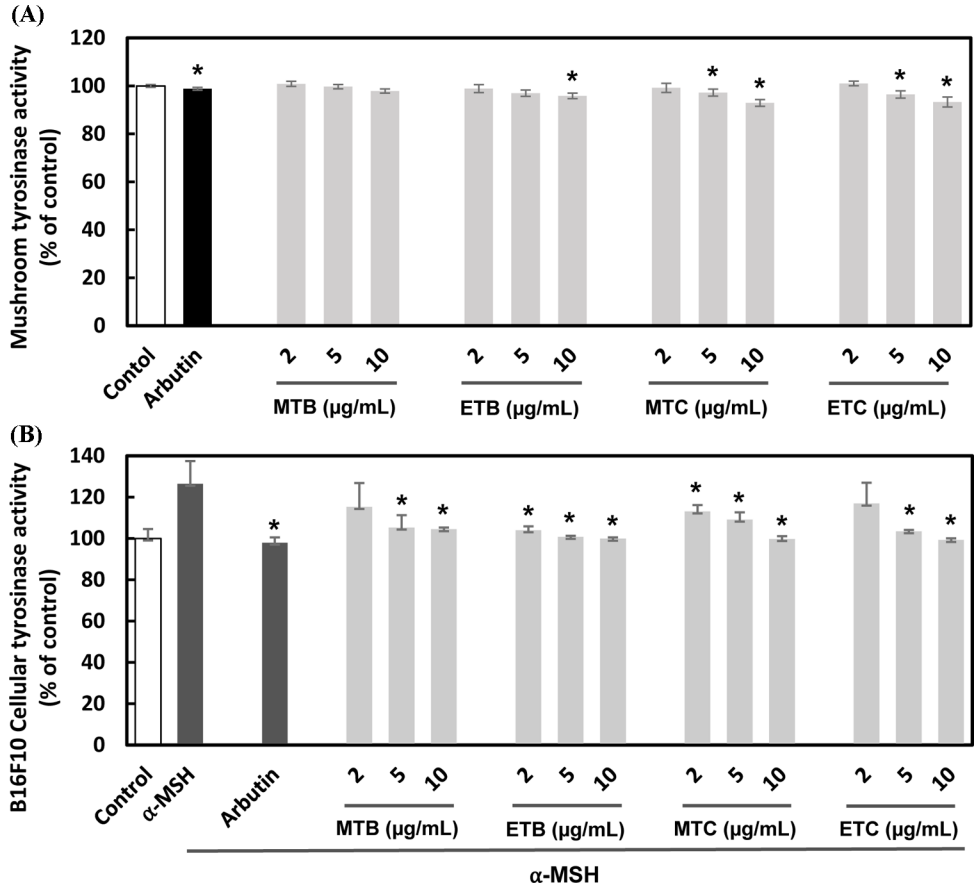

Fig. 3 Tyrosinase activation inhibition assays. (A) Mushroom tyrosinase activity and (B) B16F10 cellular tyrosinase activity. Statistical significance was compared with "Control" group for Fig. 3A and "α-MSH" group for Fig. 3B (*p <0.05). Control: not treated with α-MSH and derivative; α-MSH: 100 nM α-MSH treatment group; arbutin: 100 nM α-MSH and 125 μg/mL arbutin treatment group. MTB, methyl 3,4,5-trimethoxybenzoate; ETB, ethyl 3,4,5-trimethoxybenzoate; MTC, methyl 3,4,5-trimethoxycinnamate; ETC, ethyl 3,4,5-trimethoxycinnamate; α-MSH, alpha-melanocyte-stimulating hormone

positive control, is a well-known skin-brightening agent, which decreased α-MSH-stimulated melanin production to 83.1±2.2%. Thus, the anti-melanogenic effects of MTB (≥10 μg/mL), ETB (≥10 μg/mL), MTC (≥10 μg/mL), and ETC (≥5 μg/mL) were comparable to that of arbutin (125 μg/mL).

MTC and ETC significantly reduced the protein expression while suppressing the L-DOPA oxidase activity of mushroom tyrosinase and intracellular tyrosinase. This potent inhibition induced by trimethoxycinnamates is attributable to the previous finding that cinnamic acid and its derivatives function as noncompetitive and irreversible mushroom tyrosinase inhibitors [32]. MTB and ETB reduced the overall activity and protein expression level of intracellular tyrosinase, whereas they did not directly inhibit mushroom tyrosinase. These results comply with previous studies of methyl gallate, which has a structural similarity with MTB and CTB. Methyl gallate did not show a direct inhibition of tyrosinase activity but decreased melanogenesis in Mel-Ab melanoma cells by inducing regulation of MITF, which orchestrates the expression of tyrosinase in melanocyte [2,33]. This indicates that trimethoxycinnamates exerted direct tyrosinase inhibition, whereas trimethoxybenzoates did not induce a direct inhibition of tyrosinase activity but decreased melanogenesis.

Using ROS scavengers as alternative skin-brightening agents have received considerable attention regarding ROS-induced melanin synthesis. All derivatives considerably reduced DPPH radical molecules in a dosage- and time-dependent manner and all derivatives at a concentration of 10 μg/mL exerted strong scavenging effects on free radicals, which were approximately 2-fold higher than that of arbutin (125 μg/mL). These results indicated that derivatives of trimethoxybenzene can still effectively reduce oxidative stress even after the esterification to improve structural stability.

In conclusion, the derivatives of trimethoxybenzoate exerted anti-melanogenic and antioxidant effects on α-MSH-stimulated melanogenesis. Thus, derivatives of trimethoxybenzoate may be helpful in developing novel hypopigmenting and antioxidant agents.

Conflicts of Interest The authors declare no conflict of interest.

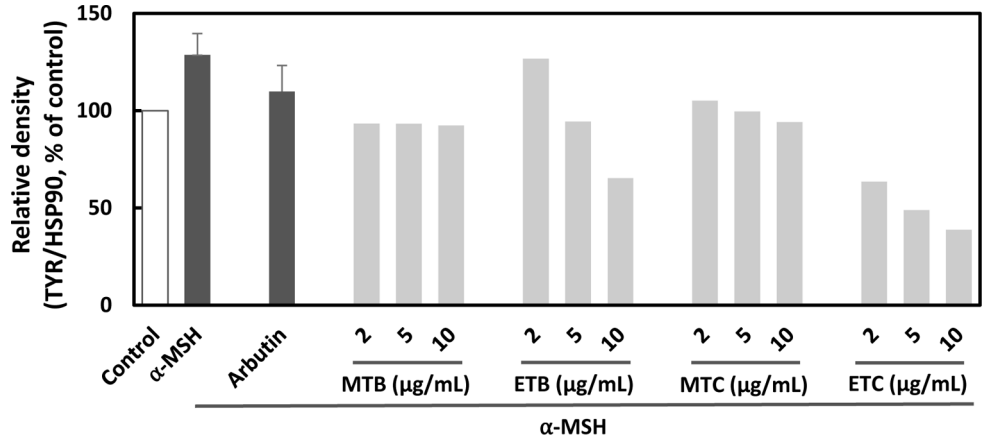

Fig. 4 Measurement of tyrosinase protein expression levels via western blotting. Tyrosinase levels were normalized to those of HSP90. Control: not treated with α-MSH and derivative; α-MSH: 100 nM α-MSH treatment group; arbutin: 100 nM α-MSH and 125 μg/mL arbutin treatment group. MTB, methyl 3,4,5-trimethoxybenzoate; ETB, ethyl 3,4,5-trimethoxybenzoate; MTC, methyl 3,4,5-trimethoxycinnamate; ETC, ethyl 3,4,5-trimethoxycinnamate; α-MSH, alpha-melanocyte-stimulating hormone; HSP90, heat shock protein 90

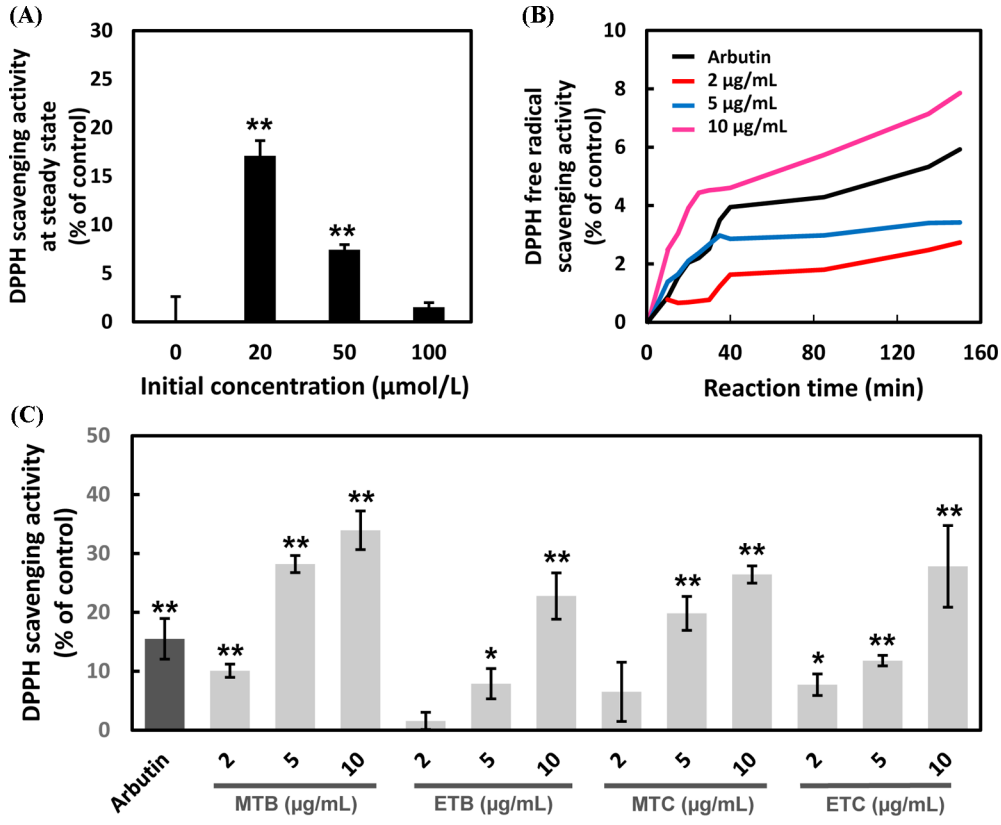

Fig. 5 DPPH scavenging activity assay. Preliminary experiments were performed to optimize DPPH experimental conditions: (A) the optimal initial DPPH concentration with ETB (10 μg/mL) and (B) the optimal reaction with DPPH (20 μmol/L) using 3B2 (2, 5, and 10 μg/mL) or arbutin (125 μg/mL). (C) Antioxidant activity of derivatives was determined using DPPH (20 μmol/L) after 80 min. Statistical significance was compared with "0" group for Fig. 5A and "Control" group for Fig. 5C (*p <0.05, **p <0.01). Control: DPPH (20 μmol/L) without derivative; arbutin: arbutin (125 μg/mL) treatment group. MTB, methyl 3,4,5-trimethoxybenzoate; ETB, ethyl 3,4,5-trimethoxybenzoate; MTC, methyl 3,4,5-trimethoxycinnamate; ETC, ethyl 3,4,5-trimethoxycinnamate; α-MSH, alpha-melanocyte-stimulating hormone; DPPH, 2,2-diphenyl-1-picrylhydrazyl

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References

1. Huang HC, Hsieh WY, Niu YL, Chang TM (2014) Inhibitory effects of adlay extract on melanin production and cellular oxygen stress in B16F10 melanoma cells. Int J Mol Sci 15: 16665–16679. doi: 10.3390/ijms150916665
2. Iozumi, K, Hoganson GE, Pennella R, Everett MA, Fuller BB (1993) Role of tyrosinase as the determinant of pigmentation in cultured human melanocytes. J Invest Dermatol 100: 806–811. doi: 10.1111/1523-1747.ep12476630
3. Bonaventure J, Domingues MJ, Larue L (2013) Cellular and molecular mechanisms controlling the migration of melanocytes and melanoma cells. Pigment Cell Melanoma Res 316–325. doi: 10.1111/pcmr.12080
4. Gilchrest BA, Park H, Eller MS, Yaar M (1996) Mechanisms of ultraviolet light-induced pigmentation. Photochem Photobiol 63: 1–10. doi:10.1111/j.1751-1097.1996.tb02988.x
5. Naeyaert JM, Eller M, Gordon PR, Park HY, Gilchrest BA (1991) Pigment content of cultured human melanocytes does not correlate with tyrosinase message level. Br J Dermatol Suppl 297–303. doi: 10.1111/j.1365-2133.1991.tb14161.x
6. Abdel-Malek Z, Swope VB, Suzuki I, Akcali C, Harriger MD, Boyce ST, Hearing VJ (1995) Mitogenic and melanogenic stimulation of normal human melanocytes by melanotropic peptides. Proc Natl Acad Sci USA 92: 1789–1793. doi: 10.1073/pnas.92.5.1789
7. Meyskens FL, Chau HV, Tohidian N, Buckmeier J (1997) Luminol-enhanced chemiluminescent response of human melanocytes and melanoma cells to hydrogen peroxide stress. Pigment Cell Res 10: 184–189. doi: 10.1111/j.1600-0749.1997.tb00482.x
8. Slominski A, Tobin DJ, ShibaharaS, Wortsman J (2004) Melanin pigmentation in mammalian skin and its hormonal regulation. Physiol Rev 84: 1155–1228. doi: 10.1152/physrev.00044.2003
9. Funasaka Y, Komoto M, Ichihashi M (2000) Depigmenting effect of alpha-tocopheryl ferulate on normal human melanocytes. Pigment Cell Res 8: 170–174. doi: 10.1111/j.0893-5785.2000.130830.x
10. Chakraborty AK, Funasaka Y, Slominski A, Ermak G, Hwang J, Pawelek JM, Ichihashi M (1996) Production and release of proopiomelanocortin (POMC) derived peptides by human melanocytes and keratinocytes in culture: Regulation by ultraviolet B. Biochim Biophys Acta 1313: 130–138. doi: 10.1016/0167-4889(96)00063-8
11. Global Industry Analysts I (2021) Skin lightening agents-global market trajectory & analytics. IOP Publishing Businesswire. https://url.kr/g47pj5. Published on April 2021
12. Huang CH, Sung HC, Hsiao CY, Hu S, Ko YS (2013) Transdermal delivery of three vitamin C derivatives by er:YAG and carbon dioxide laser pretreatment. Lasers Med Sci 28: 807–814. doi: 10.1007/s10103-012-1151-y
13. Duskova J, Dusek J, Jahodar L, Poustka F (2005) Arbutin, salicin: The possibilities of their biotechnological production. Ceska Slov Farm 54(2): 78–81
14. Zhou H, Kepa JK, Siegel D, Miura S, Hiraki Y, Ross D (2009) Benzene metabolite hydroquinone up-regulates chondromodulin-I and inhibits tube formation in human bone marrow endothelial cells. Mol Pharmacol 76: 579–587. doi: 10.1124/mol.109.057323
15. Pillaiyar T, Manickam M, Namasivayam V (2017) Skin whitening agents: Medicinal chemistry perspective of tyrosinase inhibitors. J Enzyme Inhib Med Chem 32: 403–425. doi: 10.1080/14756366.2016.1256882
16. Pan R, Kong X, Cheng Y, Du L, Wang Z, Yuan C, Cheng J, Yuan Z, Zhang H, Liao Y (2021) 1,2,4-Trimethoxybenzene selectively inhibits NLRP3 inflammasome activation and attenuates experimental autoimmune encephalomyelitis. Acta Pharmacologica Sinica 42: 1769–1779. doi: 10.1038/s41401-021-00613-8
17. Song K, An SM, Kim M, Koh JS, Boo YC (2011) Comparison of the antimelanogenic effects of p-coumaric acid and its methyl ester and their skin permeabilities. JDermatol Sci 63: 17–22. doi: 10.1016/j.jdermsci.2011.03.012
18. Su TR, Lin JJ, Tsai CC, Huang TK, Yang ZY, Wu MO, Zheng YQ, Su CC, Wu YJ (2013) Inhibition of melanogenesis by gallic acid: Possible involvement of the PI3K/akt, MEK/ERK and wnt/beta-catenin signaling pathways in B16F10 cells. Int J Mol Sci 14: 20443–20458. doi: 10.3390/ijms141020443
19. Jun H, Lee JH, Cho B, Seo W, Kim D, Cho K, Lee S (2012) p-Coumaric acid inhibition of CREB phosphorylation reduces cellular melanogenesis. Eur Food Res Technol 235: 1207–1211. doi: 10.1007/s00217-012-1830-8
20. Panich U, Onkoksoong T, Limsaengurai S, Akarasereenont P, Wongkajornsilp A (2012) UVA-induced melanogenesis and modulation of glutathione redox system in different melanoma cell lines: The protective effect of gallic acid. J Photochem Photobiol B: Biol 108: 16–22. doi: 10.1016/j.jphotobiol.2011.12.004
21. Bkowska A, Kucharska AZ, Oszmiaski J (2003) The effects of heating, UV irradiation, and storage on stability of the anthocyanin-polyphenol copigment complex. Food Chemistry 81: 349–355. doi: 10.1016/S0308-8146(02)00429-6
22. Kim IW, Jeong HS, Kim JK, Lee JK, Kim HR, Yun HY, Baek KJ, Kwon NS, Park KC, Kim DS (2015) Methyl gallate from acer barbinerve decreases melanin synthesis in mel-ab cells. Die Pharmazie: 70(1), 55–59. doi: 10.1691/ph.2015.4683
23. Choi C, Jeong HW (2020) Study on skin whitening and antioxidant effect of anemarrhenae rhizoma extract. J Physiol & Pathol Korean Med 34: 67–74. doi: 10.15188/kjopp.2020.04.34.2.67
24. Nairn R, Cresswell W, Nairn J (2015) Mushroom tyrosinase: A model system to combine experimental investigation of enzyme-catalyzed reactions, data handling using R, and enzyme-inhibitor structural studies. Biochem Mol Biol Educ 43: 370–376. doi: 10.1002/bmb.20887
25. Molagoda IMN, Choi YH, Lee S, Sung J, Lee CR, Lee HG, Lim J, Lee K, Jeon Y, Ma J, Kim G (2019) Ethanolic extract of hippocampus abdominalis exerts anti-melanogenic effects in B16F10 melanoma cells and zebrafish larvae by activating the ERK signaling pathway. Cosmetics (Basel) 7. doi: 10.3390/cosmetics7010001
26. Brand-Williams W, Cuvelier ME, Berset C (1995) Use of a free radical method to evaluate antioxidant activity. LWT-Food Sci Technol 28: 25–30. doi: 10.1016/S0023-6438(95)80008-5
27. Akaberi M, Emami SA, Vatani M, Tayarani-Najaran Z (2018) Evaluation of antioxidant and anti-melanogenic activity of different extracts of aerial parts of N. sintenisii in murine melanoma B16F10 cells. Iran J Pharm Res 17(1): 225–235
28. Micillo R, Pistorio V, Pizzo E, Panzella L, Napolitano A, D'Ischia M (2017) 2-S-lipoylcaffeic acid, a natural product-based entry to tyrosinase inhibition via catechol manipulation. Biomimetics (Basel) 2: 15. doi: 10.3390/biomimetics2030015
29. Kim JK, Park KT, Lee HS, Kim M, Lim YH (2012) Evaluation of the inhibition of mushroom tyrosinase and cellular tyrosinase activities of oxyresveratrol: Comparison with mulberroside A. J Enzyme Inhib Med Chem 27: 495–503. doi: 10.3109/14756366.2011.598866
30. Wu LH, Li P, Zhao QL, Piao JL, Jiao YF, Kadowaki M, Kondo T (2014) Arbutin, an intracellular hydroxyl radical scavenger, protects radiation-induced apoptosis in human lymphoma U937 cells. Apoptosis 19: 1654–1663. doi: 10.1007/s10495-014-1032-x.
31. Boo YC (2021) Arbutin as a Skin Depigmenting Agent with Antimelanogenic and Antioxidant Properties. Antioxidants (Basel) 10: 1129. doi: 10.3390/antiox10071129
32. Shi Y, Chen Q, Wang Q, Song K, Qiu L (2005) Inhibitory effects of cinnamic acid and its derivatives on the diphenolase activity of mushroom (agaricus bisporus) tyrosinase. Food Chemistry 92: 707–712. doi: 10.1016/j.foodchem.2004.08.031
33. Fang D (2002) Selective down-regulation of tyrosinase family gene TYRP1 by inhibition of the activity of melanocyte transcription factor, MITF. Nucleic Acids Research 30: 3096–3106. doi: 10.1093/nar/gkf424